Gene-environment Interactions between the Codon 194 Polymorphism of XRCC1 and Antioxidants Influence Lung Cancer Risk

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Abstract. *X-ray repair cross complementing group 1 (XRCC1) is a DNA repair gene whose polymorphisms appear to influence the risk of lung cancer. We explored the influence of antioxidants on the association between the codon 194 arganine to tryptophan substitution polymorphism of XRCC1 and lung cancer risk. In a case-control study nested within a cohort of tin miners the cases were those diagnosed with lung cancer over 6 years of follow-up (n = 108). Two controls, matched on age and sex, were selected for each case by incidence density sampling. Individuals with the variant Arg194Trp allele seemed to be at lower risk for lung cancer (odds ratio (OR): 0.7, 95% confidence interval (95%CL): 0.4-1.2). Furthermore, high serum α-tocopherol (OR: 0.4, 95%CL: 0.2-0.9) and retinol (OR: 0.4, 95%CL: 0.2-0.9) levels were associated with significantly reduced risk of lung cancer among individuals with the Arg194Trp variant allele, but not among individuals with the wild-type genotype. In addition, the Arg194Trp variant reduced the risk of lung cancer associated with increased serum carotenoids compared to those with the homozygous wild-type allele. Our results show that Arg194Trp XRCC1 variant modifies the association between serum antioxidants and lung cancer risk.*

X-ray repair cross complementing group 1 (XRCC1) is a protein that complexes with DNA ligase III, DNA polymerase β and poly(ADP-ribose)polymerase to repair gaps left during base excision repair (1). XRCC1 was originally discovered in radiation-sensitive mutants and assigned to the double-strand break/recombination pathway of DNA repair (2). Polymorphisms of XRCC1, which result in amino acid substitutions, may alter the function or efficiency of DNA repair and may contribute to cancer susceptibility (3-9). The genetic polymorphism of XRCC1 at codon 194 occurs at a residue that is identical in human, hamster and mouse, suggesting that this site is evolutionarily conserved (10, 11). A BCRT domain found in many proteins responsive to DNA damage with cell cycle checkpoint functions has also been identified in XRCC1 (12). Because amino acid residues at the protein-protein interfaces of multi-protein complexes and residues involved in the active sites play a role in enzyme function, it is possible that the XRCC1 polymorphism results in altered efficiency of the protein. We reported earlier that the Arg194Trp polymorphism variant appeared to be associated with a reduced risk of lung cancer (13). The goals of the current study were to explore the interactions between the Arg194Trp XRCC1 polymorphism and serum antioxidants in relation to lung cancer risk.

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Materials and Methods

Study cohort. The subjects in this study are miners in the Yunnan Tin Corporation (YTC), China. The incidence rates of lung cancer are extraordinarily high in this population. Males more than 40 years old with underground mining experience have a crude annual incidence of over one percent. Miners aged 60-64 have an incidence rate in excess of 2.5% annually. Lung cancer represents about 80% of all cancers seen annually among YTC employees and mortality from this cancer is 10-fold higher in this area than the rest of China (14). For males over 50 years old, the lung cancer incidence rate is 3-7 times higher than SEER rates for US males (15). This population has been exposed to a number of known carcinogens, including tobacco smoke, radon and arsenic (16).

A prospective cohort study of high-risk miners of the YTC was established in 1992 with annual follow-up through 1999. Eligible high-risk miners were aged ≥ 40 years, with ≥ 70 years of underground mining and/or smelting experience, and free of cancer (except for non-melanoma skin cancer)at baseline in 1992. The baseline and follow-up activities were added to an anual YTC screening of the miners ongoing since 1973. These activities included: an interview about demographic, dietary, residential, occupational and medical histories; a 24-hour food recall; chest X-ray, physical exam; and a sputum collection. The initial cohort established in 1992 had 6,259 miners and, with each annual

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screen, more miners entered the cohort as they reached the eligibility criteria, resulting in 9143 cohort members by 1997. During the anual screening in 1993 and 1994, miners were asked to provide a fasting blood specimen. The sub-cohort who had blood specimens was representative of the larger high-risk cohort and comprised about 50% of 9143 participants. Lung cancer cases were ascertained by reports to the Cancer registry of the Labor Protection Institute of the YTC or from the annual screens and were confirmed by the Joint NCI/YTC Diagnosis Review Committee. Over seventy percent of the cases indentified were classified as squamous cell carcinoma of the lung.

Selection of cases and controls. The cases consisted of 106 men and women, aged 40-74 years, diagnosed with primary lung cancer during the years 1993-1997 among the sub-cohort of those who had given blood. Using incidence density sampling, the controls were selected from cohort participants who were alive and free of cancer at the time the matched case was diagnosed. Controls were matched to cases on age (± 2 years) and sex in a 2:1 ratio. Selection of cases and controls was independent of the assessment of XRCC1 genotype.

Definition of exposures. All variables used in this study were derived from baseline evaluations. The cumulative radon exposure estimate for each subject was obtained by summing across the estimated working level months (WLM) for each job held at the YTC prior to the date of entry at initial screening for the high-risk cohort. The cumulative individual arsenic exposure for each subject was estimated by using an index for arsenic exposure (Index of Arsenic Exposure Months or IAEM), which was calculated as a time-weighted average of arsenic concentration (mg/m^3) times exposure months (mg/m^3 x months). Individuals who had smoked cigarettes and/or pipes (water pipes or Chinese long-stem pipes) regularly for 6 months or longer at any time in their life were classified as ever smokers and were asked for information on a variety of smoking-related issues. Pack year equivalents (grams/day x years ÷ 20) were used to measure cumulative tobacco onsumption, which was calculated separately for cigarettes (1 cigarette = 1 gram), water pipe, long stem pipe and for total tobacco use (17). The tobacco exposure variables used in the current study were derived from the total tobacco (grams/day) use variable and years of smoking all tobacco products.

Assessment of antioxidant levels. Serum was collected at the time of entry into the cohort (on the average 2 years prior to case diagnosis) and was analyzed in case-control triplet sets. Interpersed throughout the sets were 48 masked quality control samples (13% of study group) composed of pooled sera arranged also in sets of three to include one set per batch of analysis. Lab personnel at the NHANES laboratory of Biochemical Analyses at the Centers for the Disease Prevention and Control, Atlanta, USA, were blind to the case-control status and identity of QC samples. The intra-set coefficients of variation (based on masked reference serum assays) were 2.7, 3.7, 5.3, 1.0, 7.1, 4.6 and 2.1 for alpha-tocopherol, gamma-tocopherol, selenium, retinol, beta-carotene, lutien/zeaxanthin and beta-cryptoxanthin, respectively. The inter-batch coefficients of variation were 4.1, 3.5, 11.4, 1.1, 8.4, 3.2 and 5.1 for alpha-tocopherol, gamma-tocopherol, selenium, retinol, beta-carotene, lutien/zeaxanthin and beta-cryptoxanthin, respectively. Lycopene was not included in the analyses because the serum levels were below the detectable limits and had higher coefficients of variation than the other carotenoids. Alpha-carotene was also dropped due to the very narrow range of observed serum levels (0-12 μg/dl).

Serum levels of alpha-tocopherol, gamma-tocopherol and carotenoids were measured by isocratic high performance liquid chromatography with detection at three different wavelengths (18). Selenium was measured in the serum by atomic absorption spectrometry. Quantification was based on the measurement of light absorved at 196.0 nm by ground state atoms of selenium from a selenium electrodeless discharge lamp (EDL) source (18).

Polymorphism analyses. Polymorphism analyses were performed using genomic DNA from lymphocytes using the ABI Prism 7700 sequence detector ("TaqMan™", Applied Biosystems, Foster City, CA, USA). PCR primers and dual-labeled allele discrimination probes were designed using PrimerExpress™ version 1.0 (Applied Biosystems). Probes were selected that had a predicted T_m near 68°C, with the polymorphic base near the center. Flanking PCR primers were selected based on the calculated penalty score, T_m, length, and amplimer size. For the C to T polymorphism in exon 6, codon 194 "turbo" probes with T = 5-propyne-2'-deoxyuridine were used. Oligonucleotide sequences for the analyses were:

Exon 6 codon 194:

Forward Primer: GAGGATGAGAGCGCCAACTCT
Reverse Primer: ACGTTGTCCGAGCTCACCTG
C allele probe: TCTTCTTCAGCCGGATCAACAAG
T allele probe: CTCTTCTTCAGCTGGATCAACAAGA

Genotyping reactions (10 μl) contained approximately 20 ng of genomic DNA, 1x TaqMan™ Master Mix, dual-labeled probes (100 nM each), and PCR primers (900 nM each). Reactions were performed in 96-well MicroAmp® Optical reaction plates and caps (PE Biosystems).Plates were incubated at 50°C for 2 minutes, 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 64°C for 1 minute. Reaction data was analyzed with Sequence Detection System version1.6.3. Amplified DNA from several individuals exhibiting each genotype was electrophoresed on an agarose gel to confirm amplimer size and sequenced to confirm each genotype. All lab personnel were blind to the case-control status of the samples. For quality control, genotype determinations were repeated for a random sample of 10% of study participants and we observed a 100% concordance rate.

Statistical analyses. The Wilcoxon rank sum test was used to test the hypothesis that the distribution of baseline characteristics was the same for cases and controls. The Chi-square test was used for categorical variables to test the hypothesis that the distribution of allele prevalences was the same for cases and controls. Conditional logistic regression analyses were used to examine the association between genotype and lung cancer risk. Modification of the effect of genotype on the odds of lung cancer by serum analytes (p-values for interactions) were examined by statistical tests of the first order interaction term in the logistic regression models. Linear trend analyses were conducted by creating variables using exposure scores based on the median values of each metabolite for the first to third tertiles among the controls. Analyses stratified by antioxidants were conducted by breaking the case-control match to avoid the loss of subjects due to splitting of matched sets that fell into different strata and using unconditional logistic regression adjusted for age and sex (the original matching criteria) and other potential confounders. Tertiles for antioxidants were created using the distribution of individual antioxidants among the controls.

Potential confounding of the association between genotype and cancer risk by other related risk factors was explored using Spearman rank correlation analysis and multivariate logistic regression models, including stepwise regression models both before and after stratificaiton. If the potential confounder caused a significant change in the log likelihood estimate ($p < 0.05$) and a greater than 20% change in the beta-coefficient, it was kept in the model for further multivariate analysis. Exclusion of early cases (diagnosed within one year of blood draw) did not materially alter any of the risk estimates. All analyses were performed using the statistical software package STATA (STATA Corporation, College Station, TX, USA).

Results

The median age of cases and controls was 63 years and most were retired (99%). Comparison of lifestyle and occupational variables that could be related to cancer risk yielded some

Table I. *Risk factors for lung cancer comparing cases to controls*[1].

Risk Factor	Cases (n = 108)	Controls (n = 216)	*p*-value[2]
Age (Years)	63	63	0.88
All tobacco smoked (grams/day)	17	13	< 0.01
Years of all tobacco smoking	45	43	< 0.05
Pack year equivalents (all tobacco)	34	25	< 0.01
Alcohol (grams/day)			
All men	5	0	0.18
Drinkers (48%)	180	171	0.39
Cumulative radon exposure [WLM]	570	408	< 0.05
Cumulative arsenic exposure [IAEM]	11,961	10,101	0.30
Years mining/smelting jobs	31	29	< 0.05
Serum			
Alpha Tocopherol (ug/dl)	778	773	0.64
Gamma Tocopherol (ug/dl)	102.5	101.5	0.97
Selenium (ng/ml)	46.5	445.0	0.75
Retinol (ug/dl)	49.0	51.5	0.12
Lutein & Zeaxanthin (ug/dl)	55	52	0.92
Beta Carotene (ug/dl)	16.0	13.5	0.14
Beta-Crytoxanthin (ug/dl)	7	6	0.07
Exon 6, codon 194			
Arg/Arg	52 (48%)	85 (40%)	
Arg/Trp	47 (44%)	104 (50%)	0.42
Trp/Trp	9 (8%)	21 (10%)	
Arg/Trp + Trp/Trp	56 (52%)	125 (60%)	0.19

[1]Based on unmatched data with continuous variables expressed as the median and counts for gentotypes.
[2]*p*-values as determined by Wilcoxon rank-sum tests and χ^2 for genotype.

differences between cases and controls (Table I). As expected, tobacco use and radon exposure were significantly higher among the cases compared to the controls. The cases also performed mining and smelting work longer than the controls.

Prediagnostic serum antioxidant levels by case status is also shown in Table I. Alpha-tocopherol, gamma-tocopherol, selenium and retinol levels did not differ by case-control status. The serum alpha-tocopherol, gamma-tocopherol, selenium and retinol levels ranged from 440-1658 μg/dl, 44-425 μg/dl, 20-111 ng/ml and 13-117 μg/dl, respectively, among the controls while the serum alpha-tocopherol, gamma-tocopherol, selenium and retinol levels ranged from 316-1850 μg/dl, 40-420 μg/dl, 22-121 ng/ml and 23-105 μg/dl, respectively, among the cases. All the carotenoid levels were marginally higher among the cases than controls, but were not statistically significant. The serum carotenoid levels ranged from 1-64, 10-123 and 0-41 μg/dl for beta-carotene, lutien/zeaxanthin and beta-cryptoxanthin, respectively, among the controls, while the serum carotenoid levels ranged from 1-90, 14-147 and 1-80 μg/dl for beta-carotene, lutien/zeaxanthin and beta-cryptoxanthin, respectively, among the cases. Table I shows also the distribution of XRCC1 variant alleles by case-control status.

Table II. *Lung cancer risk & antioxidant status stratified by XRCC1 Arg194Trp genotype*[1]

	Overall	WT: Arg/Arg[2]	VT: Arg/Trp + Trp/Trp[2]
	OR (95% CI) [Cases/Controls]	OR (95% CI) [Cases/Controls]	OR (95% CI) [Cases/Controls]
Overall:		1.0 (ref) [52/85]	0.7 (0.4 - 1.2) [56/125]
Alpha tocopherol (μg/dl):			
Tertile 1: < 706	1.0 (ref) [40/72]	1.0 (ref) [12/30]	1.0 (ref) [28/42]
Tertile 2: 707 - 892	1.3 (0.7 - 2.4) [38/71]	2.2 (0.9 - 5.8) [21/28]	0.7 (0.3 - 1.4) [17/40]
Tertile 3: > 893	1.1 (0.6 - 2.3) [30/73]	2.4 (0.9 - 6.2) [19/27]	0.4 (0.2 - 0.9) [11/43]
	P_{trend} = 0.29	P_{trend} = 0.3	P_{trend} = 0.02
		$P_{interaction}$ = 0.01	
Gamma tocopherol (μg/dl):			
Tertile 1: < 85	1.0 (ref) [33/72]	1.0 (ref) [9/33]	1.0 (ref) [24/37]
Tertile 2: 86 - 120	1.1 (0.6 - 2.1) [37/72]	3.0 (1.1 - 8.0) [22/28]	0.5 (0.2 - 1.2) [15/43]
Tertile 3: > 121	1.4 (0.7 - 2.7) [38/72]	3.8 (1.4 - 10.6) [21/24]	0.6 (0.3 - 1.2) [17/45]
	P_{trend} = 0.62	P_{trend} = 0.02	P_{trend} = 0.2
		$P_{interaction}$ = 0.06	
Selenium (ng/ml):			
Tertile 1: < 39	1.0 (ref) [31/69]	1.0 (ref) [18/24]	1.0 (ref) [13/44]
Tertile 2: 40 - 54	1.2 (0.6 - 2.3) [38/74]	0.9 (0.3 - 2.2) [18/26]	1.5 (0.6 - 3.3) [20/46]
Tertile 3: > 55	1.2 (0.6 - 2.4) [39/73]	0.6 (0.3 - 1.6) [16/35]	2.2 (0.9 - 5.0) [23/35]
	P_{trend} = 0.52	P_{trend} = 0.4	P_{trend} = 0.08
		$P_{interaction}$ = 0.03	
Retinol (μg/dl):			
Tertile 1: < 42	1.0 (ref) [38/71]	1.0 (ref) [16/34]	1.0 (ref) [22/36]
Tertile 2: 43 - 59	1.1 (0.6 - 1.9) [43/74]	2.0 (0.8 - 5.1) [19/26]	0.9 (0.4 - 1.8) [24/46]
Tertile 3: > 60	0.7 (0.4 - 1.3) [27/71]	1.7 (0.7 - 4.1) [17/25]	0.4 (0.2 - 0.9) [10/43]
	P_{trend} = 0.7	P_{trend} = 0.4	P_{trend} = 0.03
		$P_{interaction}$ = 0.03	
Lutein & Zeaxanthine (μg/dl):			
Tertile 1: < 44	1.0 (ref) [39/75]	1.0 (ref) [13/29]	1.0 (ref) [26/44]
Tertile 2: 45 - 60	1.0 (0.5 - 2.0) [30/67]	1.1 (0.5 - 2.8) [14/28]	0.7 (0.3 - 1.5) [16/39]
Tertile 3: > 61	1.3 (0.7 - 2.4) [39/74]	2.0 (0.9 - 4.6) [25/28]	0.6 (0.3 - 1.2) [14/42]
	P_{trend} = 0.96	P_{trend} = 0.1	P_{trend} = 0.1
		$P_{interaction}$ = 0.03	
Beta Carotene (μg/dl):			
Tertile 1: < 9	1.0 (ref) [31/82]	1.0 (ref) [12/36]	1.0 (ref) [19/48]
Tertile 2: 10 - 18	1.3 (0.7 - 2.5) [34/67]	1.9 (0.8 - 4.8) [16/25]	1.1 (0.5 - 2.3) [18/40]
Tertile 3: 19 - 90	2.0 (1.1 - 3.8) [43/67]	3.0 (1.3 - 7.1) [24/24]	1.1 (0.5 - 2.4) [19/40]
	P_{trend} = 0.08	P_{trend} = 0.02	P_{trend} = 0.8
		$P_{interaction}$ = 0.09	
Beta-cryptoxanthine (μg/dl):			
Tertile 1: < 4	1.0 (ref) [25/71]	1.0 (ref) [9/34]	1.0 (ref) [16/36]
Tertile 2: 5 - 7	1.8 (0.9 - 3.8) [34/70]	2.6 (1.0 - 6.8) [18/26]	0.8 (0.4 - 1.9) [16/43]
Tertile 3: > 8	2.9 (1.4 - 5.8) [49/75]	3.8 (1.5 - 9.5) [25/25]	1.2 (0.5 - 2.5) [24/46]
	P_{trend} = 0.03	P_{trend} = 0.01	P_{trend} = 0.7
		$P_{interaction}$ = 0.07	

[1]All unmatched logistic regression models adjusted for age at baseline, radon and pack years of tobacco exposure
[2]WT: homozygous wild-type, VT: homozygous and heterozygous variant
[3]P_{trend}: *P*-value for trend
[4]$P_{interaction}$: *P*-value for interaction

Table II also shows the association between the antioxidants and cancer risk. The only statistically significant associations were between increased serum beta-carotene, beta-cryptoxanthine and lung cancer risk. Table II also shows the association between the various antioxidants and lung cancer risk stratified by genotype. Among individuals with the protective variant genotype serum alpha-tocopherol and retinol showed a significant dose response reduction in cancer risk. Compared to those in the lowest tertile of alpha-tocopherol and retinol, those in the highest tertile were at reduced risk of lung cancer. Significant positive relationships with lung cancer risk were observed for both serum gamma-tocopherol (OR3 3.8, 95% CI 1.4-10.6 for individuals in highest tertile compared to lowest tertile), beta-carotene (OR 3.0, 95% CI 1.3-7.1) and beta-cryptoxanthin (OR 3.8, 95% CI 1.5-9.5) (Table II) among those with the wild-type genotype. The variant allele apparently counteracted the adverse association between serum gamma-tocopherol, beta-carotene or beta-cryptoxanthin and lung cancer risk.

Discussion

DNA repair systems act to maintain genomic integrity in the face of environmental insults, cumulative effects of age and general DNA replication errors. XRCC1 is thought to play a role in the multi-step base excision repair pathway where "non-bulky" base adducts produced by methylation, oxidation, reduction, or fragmentation of bases by ionizing radiation or oxidative damage are removed (19). Although the specific function of XRCC1 has not been identified, it is believed that XRCC1 complexes with DNA ligase III *via* a BRCT domain in its carboxyl terminus and with DNA polymerase in its amino terminus to repair gaps left during base excision repair (20).

The Arg194Trp amino acid substitution resides in the linker region separating the DNA polymerase β domain from the poly(ADP-ribose) polymerase-interacting domain. We reported earlier that individuals with the Arg194Trp substitution allele seemed to be at reduced risk of lung cancer although the risk estimates were not statistically significant (OR: 0.7; 95% Confidence Interval: 0.43 - 1.16). This finding is consistent with that reported by Sturgis *et al.* (21) in squamous cell oral and pharyngeal cancer (OR: 0.41; 95% CL: 0.20-0.82), Shen *et al.* (22) in gastric cardia cancer (OR: 0.54; 95% CI: 0.31-0.91) and Stern *et al.* (23) in bladder cancer (OR: 0.59; 95% CL: 3 - 1.0).

The XRCC1 Arg194Trp polymorphism appears to play a protective role in perhaps reversing genomic damage. In the current study we observed statistically significant interactions between alpha-tocopherol, retinol and the Arg194Trp polymorphism. Stratified analysis revealed that higher serum alpha-tocopherol and retinol levels seemed to be protective against lung cancer among those with the Arg194Trp variant allele, while the same does not appear to be the case for those with homozygous wild-type genotype. One explanation of this finding is that the Arg194Trp amino acid substitution may be able to increase the base excision repair activity of XRCC1 compared to wild-type and compliment the protective effects of alpha-tocopherol and retinol.

The Arg194Trp polymorphism also modified the effect of carotenoids and lung cancer risk. Among the YTC study participants, higher serum carotenoid levels were associated with increased risk of lung cancer. (24). The Arg194Trp variant allele seems to reduce the risk of lung cancer associated with higher serum carotenoid levels. The Arg194Trp variant may be able to enhance DNA repair activity consequently reducing the risk of lung cancer associated with higher serum levels of beta-carotene and beta-cryptoxanthine.

Another potential explanation of our findings is that it is spurious. However, we did not observe similar interactions with the other polymorphisms or even other XRCC1 polymorphisms. The observation that the variant allele enhances the protective tendencies of retinol or alpha-tocopherol and counters the adverse association of serum beta-carotene or beta-cryptoxanthine points to consistency in the biological behavior of the Arg194Trp XRCC1 variant.

One of the strengths of this study is its prospective design. The collection of covariate data before case diagnosis minimized the potential for recall bias for measures of environmental exposures and the availability of these data also allowed us to explore gene-environment interactions. The limitations of this exploratory study includes its rather small sample size and the multiple comparisons. The generalizability of these results may also be somewhat restricted because the study was conducted among a unique group of tin miners. Larger studies examining the interaction of the XRCC1 polymorphism with antioxidants is needed.

In summary, we observed that the XRCC1 Arg194Trp variant modified the effects of serum antioxidants on lung cancer risk.

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